BILIRUBIN FLUORESCENCE. AN EXPERIMENTAL STUDY

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Abstract. The fluorescence of bilirubin (BR) was studied in the presence of different concentrations of bovine serum albumin (BSA). It was also investigated the influence of sucrose concentration on the bilirubin fluorescence.

Key words: bilirubin, fluorescence, bovine serum albumin, neonatal jaundice.

INTRODUCTION

The excited states of bilirubin (Fig. 1) are of great importance because of their relevance for the understanding of the molecular mechanisms implied in the phototherapy of newborn with physiologic jaundice [5]. After the excitation of BR from the ground state (S_0) to its first excited singlet (S_1), it can decay to S_0 with fluorescence emission.

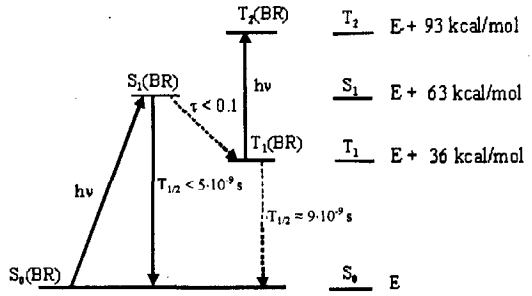

Fig. 1. – Electronic excitation of ground state bilirubin (S_0) to its first excited singlet (S_1) that can decay to S_0 with fluorescence.

It is to be noticed that the attempt of Alexander Cu and collab. [3] to evidence the fluorescence of free bilirubin in solution did not succeed. Later, it was phototherapy that emphasized that BR gives a fluorescence emission only when

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bound to different serum albumins (human, bovine, dog, goat, pig) [1, 2]; it was observed an enhancement of the fluorescence emission as the viscosity of the solution increases [4]. The detection of the fluorescence of BR bound to serum albumins is relevant for the efficiency of the newborn jaundice phototherapy. The irradiation with visible light results in bilirubin degradation. The photoproducts do not emit at the same λ as BR (λ = 550 nm).

MATERIALS AND METHODS

All reagents were of analytic purity. Bilirubin and bovine serum albumin were purchased from Merk, Germany, dioxane and sucrose from Fluka, Switzerland; potassium monophosphate and diphosphate from Reactivul, Bucharest, Romania.

The fluorescence spectra were recorded with an Aminco-Bowman spectrofluorimeter, at room temperature, with excitation at λ = 465 nm. Data acquisition in digital format was performed with AUTOLAB software running on a 486-type PC linked to the spectrofluorimeter.

RESULTS

A typical fluorescence spectrum of BR (2 μM) with 15 μM BSA in solution is presented in Figure 2.

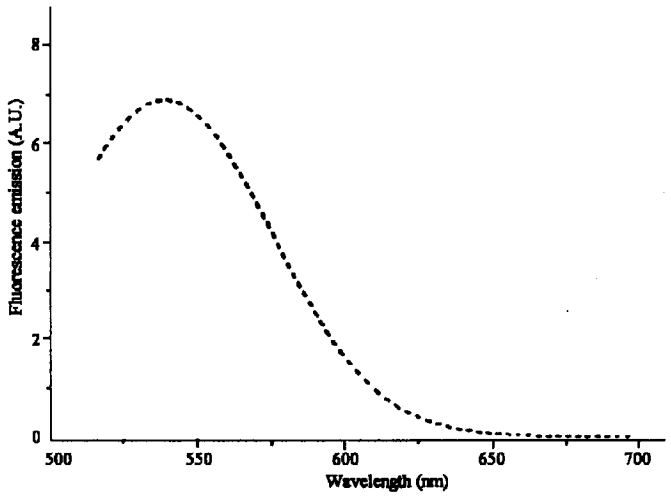

Fig. 2. – Fluorescence spectrum of bilirubin (2 μM) in the presence of BSA (15 μM).

BR FLUORESCENCE WITH 1.5 μM BSA

There were prepared solutions of BR in dioxane and mixed in phosphate-buffered solutions, pH = 7.5, with 1.5 μM BSA. The final concentrations in the test-tubes were: 0.5, 1, 2, 4, 6, 10, and 14 μM. The fluorescence emission (λ = 550 nm), in arbitrary units (A.U.), function of the BR concentration is represented in Figure 3.

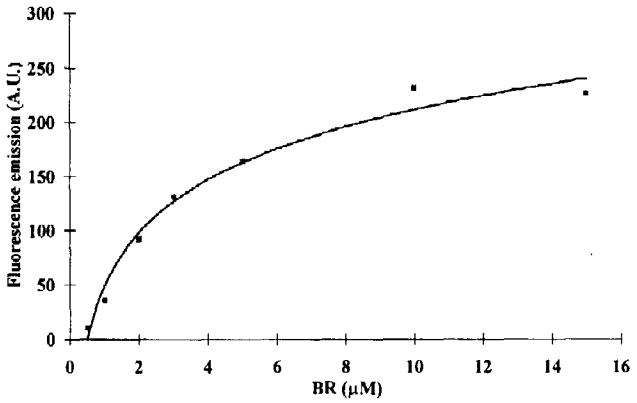

Fig. 3. – Fluorescence emission of BR in solution with 1.5 μM BSA.

BR FLUORESCENCE WITH 15 μM BSA

The solutions were prepared in the same conditions, but with a BSA concentration of 15 μM. The results are shown in Figure 4.

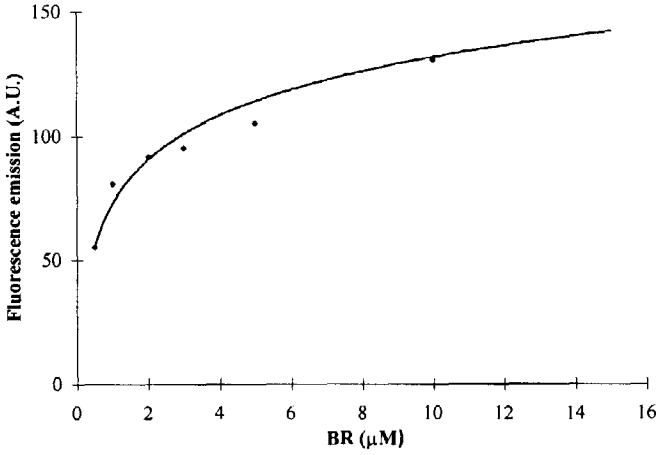

Fig. 4. – Fluorescence emission of BR in solution with 15 μM BSA.

The comparative results for both BSA concentrations are presented in Fig. 5.

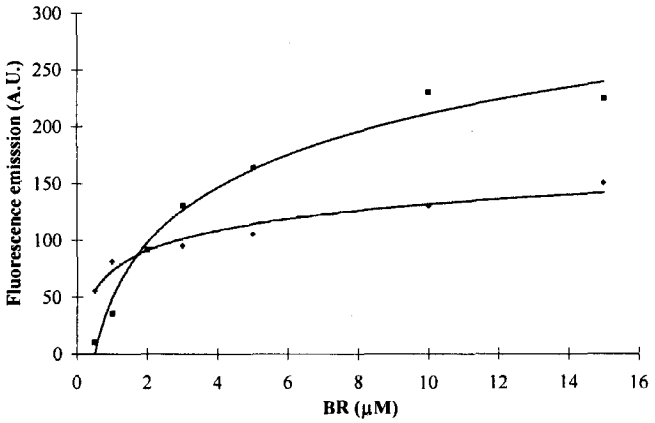

Fig. 5. – Fluorescence emission of BR for both BSA concentrations;
■ = 1.5 μM BSA; ◆ = 15 μM BSA.

BR FLUORESCENCE WITH 15 μM BSA IN THE PRESENCE OF VARIABLE SUCROSE CONCENTRATION

To the solutions prepared as above, with a constant BR concentration (2.5 μM), with 15 μM BSA, there were added various amounts of sucrose (20, 40, 60, 80%). The fluorescence spectra recorded from these solutions are shown in Fig. 6.

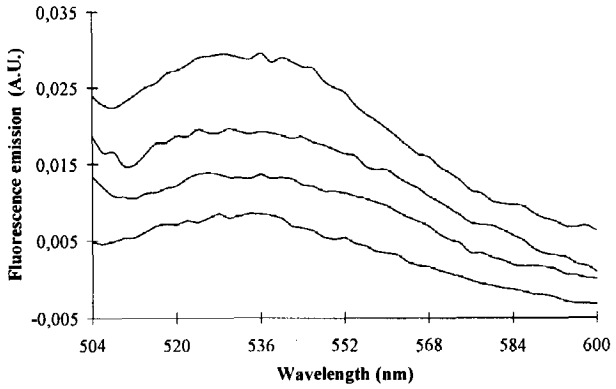

Fig. 6. – Fluorescence spectra of BR (2.5 μM) in 15 μM BSA solutions, at different sucrose concentrations: 20, 40, 60 and 80%, presented in the order of increasing amplitudes.

The fluorescence emission is enhanced as the sucrose concentration increases, as it can be seen in Figure 7.

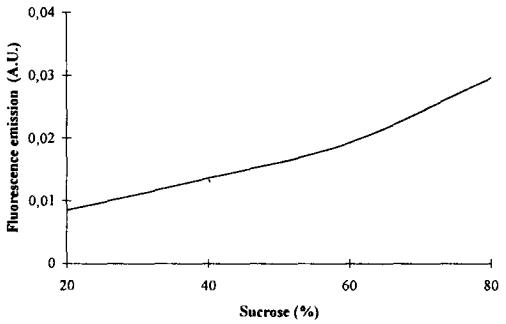

Fig. 7. – Fluorescence emission of BR (λ = 550 nm) function of sucrose concentration.

DISCUSSION

As it results from Figures 3 – 5, at both BSA concentrations, above a certain BR concentration, the fluorescence emission remains constant. Taking into account previous studies [3, 6] and the fact that, at room temperature, the bilirubin has fluorescence emission only bound to serum albumins [1], it results that the number of binding sites of BR to serum albumin is limited. By this way, we can explain why, *in vivo*, when the BR concentration in the blood exceeds a certain value, the surplus of BR cannot bind the serum albumin any more. The free BR, being liposoluble, can enter through the lipid bilayer of different cells. It results in severe damages, especially in nervous cells, as in the case of newborn jaundice. The BR liposolubility explains also its interaction with the erythrocyte membrane [7, 8].

In the same figures, it can also be seen that the fluorescence emission enhances with the increase of the amount of BR bound to the serum albumin.

Because at equal BR concentrations, the fraction of bound BR is greater in the solution containing more BSA, we could expect that the fluorescence emission would be greater in this case. But, we can see in Figure 5, this is the case only until 2 μM BR. This means that, at greater bilirubin concentration, in the solution with 15 μM BSA, the phenomenon of fluorescence quenching by concentration is produced.

In Figures 6 and 7, we can see that, in spite of the fact that the shape of the curves is the same, the fluorescence emission is enhanced as the sucrose concentration increases. Because all the other conditions were the same as before, the only difference consists in an increased viscosity at greater sucrose concentrations.

In conclusion, from our study evidences results that the fluorescence emission of BR supposes its binding to serum albumin and it is enhanced as the medium viscosity increases.

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